

## Planktotrons: A novel indoor mesocosm facility for aquatic biodiversity and food web research

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### Abstract

We established a new indoor mesocosm facility, 12 fully controlled “Planktotrons”, designed to conduct marine and freshwater experiments for biodiversity and food web approaches using natural or artificial, benthic or planktonic communities. The Planktotrons are a unique and custom-tailored facility allowing long-term experiments. Wall growth can be inhibited by a rotating gate paddle with silicone lips. Additionally, temperature and light intensity are individually controllable for each Planktotron and the large volume (600 L) enables high-frequency or volume-intense measurements. In a pilot freshwater experiment various trophic levels of a pelagic food web were maintained for up to 90 d. First, an artificially assembled phytoplankton community of 11 species was inoculated in all Planktotrons. After 22 d, two ciliates were added to all, and three *Daphnia* species were added to six Planktotrons. After 72 d, dissolved organic matter (DOM, an alkaline soil extract) was added as an external disturbance to six of the 12 Planktotrons, involving three Planktotrons stocked with *Daphnia* and three without, respectively. We demonstrate the suitability of the Planktotrons for food web and biodiversity research. Variation among replicated Planktotrons ( $n = 3$  minimum) did not differ from other laboratory systems and field experiments. We investigated population dynamics and interactions among the different trophic levels, and found them affected by the sequence of ciliate and *Daphnia* addition and the disturbance caused by addition of DOM.

Enclosed experimental systems provide a highly valuable and widely used approach bridging small-scale laboratory experiments and large-scale field surveys (Petersen et al. 2010). Until now, most small-scale laboratory experiments investigating long-term dynamics or trophic interactions are performed in chemostats or Erlenmeyer flasks with relatively small (sample) volumes (Jürgens et al. 1997; Fussmann et al. 2000; Huisman et al. 2002; Yoshida et al. 2003; Becks et al. 2010; Hardenbicker et al. 2015). While such highly controlled small-scale systems have the advantage of high replicability, they often suffer from stochastic effects, which arise from small population sizes in small sample volumes (Petersen et al. 2010). Also, the possibility to investigate volume-intense parameters is naturally limited in small-scale

setups. This particularly complicates investigations of food webs, where features of the whole community or multiple trophic levels are of interest as predictors and/or responses, often including the necessity to cover dynamic interactions over time by repeated sampling. Over longer time periods (months to years), such dynamics can be resolved by monitoring of real ecosystems in the field and whole-system experiments, with the advantage of increased realism, yet missing opportunities for manipulation, replication, or isolated investigation of mechanisms. Experimental mesocosms, indoor as well as outdoor, offer a good compromise of the two extremes (Petersen et al. 2010). Mesocosm setups offer the possibility of large-volume samples and high-frequency assessments of different abiotic and biotic parameters. Natural communities, assembled of species with a shared evolutionary history, can be used and a multitude of parameters can be manipulated, such as light (Dickman et al. 2008), temperature (Berger et al. 2006; Velthuis et al. 2017), nutrient content (Joint et al. 2002), humic content (Hansson

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et al. 2013), CO<sub>2</sub> enrichment (Riebesell et al. 2008; Verschoor et al. 2013), and predator–prey interactions by adding fish (Shurin et al. 2012). As outdoor mesocosms lack possibilities for control of some parameters and are exposed to natural disturbances and environmental conditions, the development of indoor mesocosm facilities is currently on the rise.

In Europe, especially at GEOMAR, Helmholtz-Zentrum für Ozeanforschung in Kiel (Germany), at the Umeå-Hörnefors Marine Sciences Centre (UMF) (Sweden), and at the Netherlands Institute of Ecology (NIOO) in Wageningen (Netherlands) indoor mesocosms were established and are in use. Each of these facilities has a unique design catering for different experimental purposes, with different advantages. The 12 indoor mesocosms in Kiel are suited for pelagic and benthic research, with temperature controlled for all mesocosms via room temperature. In contrast, we can regulate temperature of each one of our mesocosms separately, including separate manipulation of individual water layers within each mesocosm. Furthermore, experiments in Kiel are limited to a maximum duration of 7–8 weeks as wall growth becomes prevalent after this experimental duration (Sommer et al. 2006). On the contrary, the nine indoor mesocosms in Wageningen are equipped with a rotating paddle preventing wall growth, have light and temperature control and are thus suited for long-term experiments, yet they do not allow crossed factorial designs (e.g., 2 × 2) with replication (Verschoor et al. 2003). The 12 large (5 m high, 0.8 m diameter) indoor mesocosms in Umeå allow computer-controlled temperature and light regulation and thus provide the possibility to simulate all types of environmental conditions in large-scale experimental setups (Berglund et al. 2007; Grubisic et al. 2012; Lefébure et al. 2013).

To create realistic conditions for food web and biodiversity research in the context of global change we wanted to establish a new indoor mesocosm facility, which combines advantages of already existing mesocosms with unique custom-tailored features. We envisioned an experimental infrastructure mimicking a pelagic environment, where we can readily manipulate multiple key abiotic parameters. The latter include temperature and light, water constituent-related factors such as pH and concentrations of nutrients and organic matter, as well as physical disturbance (mixing). The facility should be suited for both freshwater and marine experiments and should allow control of the biotic composition of the plankton: experiments may rely on a controlled inoculation of cultured species or may use a natural plankton assemblage. Temperature-control must not only be able to increase mean temperature, but should also be capable to alter its temporal variance and vertical stratification. Enhanced stratification may be a major pathway linking climate change-altered temperature to primary production, as increased stability of the water body reduces the nutrient input into the upper mixed layer and thereby curbs

phytoplankton growth (Behrenfeld et al. 2006). Light sources should allow independent treatments of quantity (irradiance) and quality (spectral wavelength distribution) in realistic natural ranges. The manipulation of pH can always be achieved by direct manipulation of water chemistry, and our facility should allow to create changes in aquatic concentrations of CO<sub>2</sub> via atmospheric enrichment or aeration, thus mimicking realistic ocean acidification scenarios. And last, we wished to efficiently counteract wall growth, which often limits the duration of mesocosm experiments, as measured (Chen et al. 1997).

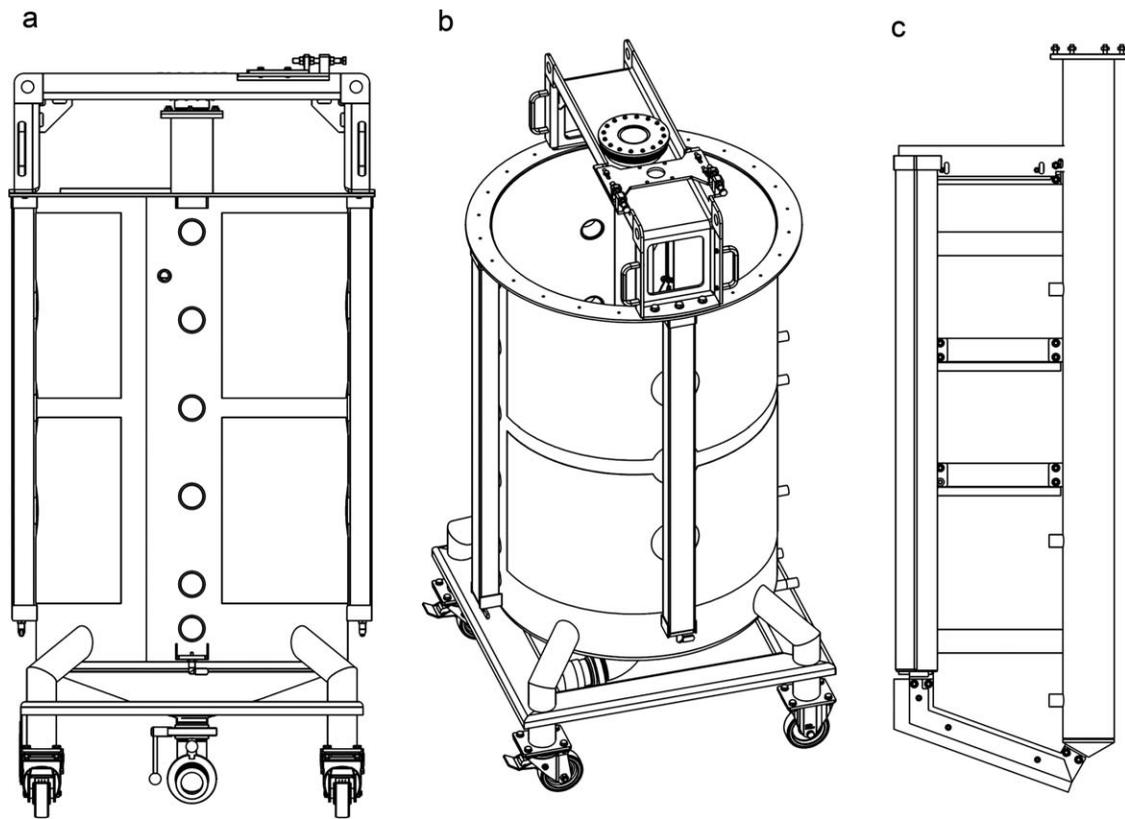
Here, we describe the technical properties of the “Planktotrons”, which allow this broad array of approaches. Each of the 12 Planktotrons offers full control of environmental conditions such as light and temperature, and allows surveillance of desired nutrient conditions and of a target species composition. Their large size (600 L) allows the simultaneous determination of several volume-intensive measures at high frequency and over several months at near-natural conditions. The large size also allows sufficiently large population sizes at higher trophic levels, thus minimizing stochastic effects arising from small population sizes. We included rotating paddles inside the mesocosms to automatically remove wall growth via silicon lips. The paddles are divided in segments, which are individually removable and allow us to conduct either benthic or pelagic experiments, or a combination of both. The consistent use of high-grade stainless steel allows the use of saltwater up to full marine conditions. Glass plates that cover the mesocosms reduce evaporation and—following simple hardware modification—may create gas-tight setups if needed. In summary, our mesocosms offer a unique combination of advantages: they are suited for pelagic and benthic experiments, for long term-experiments and for replicated factorial designs.

As a proof of concept, we examined the development and response of a freshwater phytoplankton community, ciliates, and various *Daphnia* species under an external stressor exemplified by the sudden addition of a sizeable amount of allochthonous colored dissolved organic matter. Primarily, we wanted to test the suitability of the Planktotrons for food web and biodiversity research by examining among-system variance and comparability to other laboratory systems and field data. However, our investigation also addresses ecological questions as our examination specifically included an assessment of (1) population dynamics and interactions between the different trophic levels, and (2) disturbance effects caused by the addition of dissolved organic matter.

## Materials and procedures

### General description of the experimental facility, the Planktotrons

The experimental facility consists of 12 indoor mesocosms, so-called Planktotrons. These indoor mesocosms are



**Fig. 1.** Technical drawing of a Planktotron. Each Planktotron (a) has a total height of 1.93 m (including the substructure and the strap with the motor on top). The inner height is 1.2 m. The outer and inner diameters measure 0.95 m and 0.80 m, respectively, enclosing a volume of 600 L. Sampling ports are installed at the front side at 0.01 m, 0.33 m, 0.56 m, 0.79 m, 1.01 m, and 1.13 m below the top margin (a). Additional ports are installed at the left and right sides of the Planktotrons (at 0.29 m, 0.56 m, and 0.82 m below the top margin). The tanks are double-walled pillow plates to control the temperature of each Planktotron in three different zones (a, b): the upper zone, the lower zone, and the bottom zone. Three temperature sensors are installed in the rotating paddle at 0.30 m (upper temperature zone), 0.83 m (lower temperature zone), and 1.16 m (bottom zone) below the top margin (c). The paddle is fixed at the top with the strap and connected to the motor. Wipers (silicone lips) at the bottom, the side and the top of the paddle remove wall growth and precipitated water from the glass plates.

custom-tailored units (Klarmann Edelstahl Technik, Westerstede, Germany) that were developed and established at the University of Oldenburg, ICBM Wilhelmshaven. The mesocosms were set up in a room assuring constant and controllable temperature conditions through heat recovery-ventilation (Santos 370 DC, PAUL, Reinsdorf, Germany) with a brine-air heat exchanger (SD 500, PAUL, Reinsdorf, Germany) and a downstream cooling coil (AVA 200, Salda, Šiauliai, Lithuania). Room temperature can be regulated down to 18°C as long as outside temperature is less than 25°C.

The Planktotrons are built of austenitic stainless steel (steel-no. 1.4404, EN X2CrNiMo17-12-2/AISI 316L), which was used for all surfaces that could possibly be in contact with sea- or freshwater medium. Steel-no. 1.4301 (EN X5CrNi18-10/AISI 304) was used for surfaces not in contact with medium. Each tank has a fillable height of 1.2 m and an inner diameter of 0.8 m (Fig. 1), resulting in a volume of 600 L. The two-walled tank with pillow-plate technology

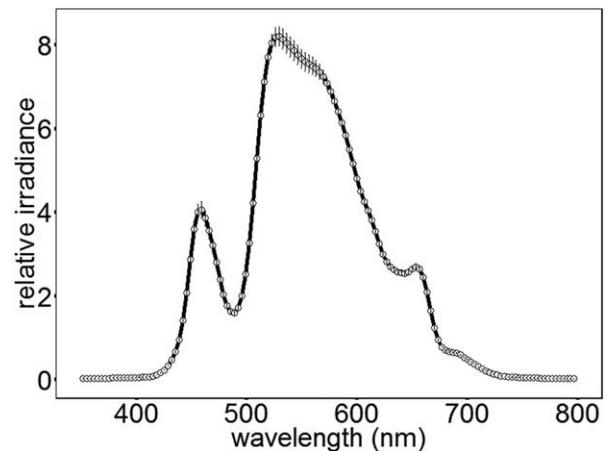
allow temperature control independently in three vertical strata (Fig. 1) by circulating heating or cooling medium between the two walls. Temperature is measured using PT100 sensors (Temperature Control, Donaueschingen, Germany) mounted on the rotating paddle at three different depths (0.30 m, 0.83 m, and 1.16 m below the top margin, thus in each temperature zone) (Fig. 1). The temperature is controlled by an ATMega 2561 (Atmel AVR) based microcontroller board that regulates thermostatic valves (TA Heimeier V-exact II, Erwitte, Germany) by thermal actuators (Alpha 5, Möhlenhoff, Salzgitter, Germany), which determine the amount of heating or cooling medium flowing through heat exchanging devices. Each temperature unit (one per temperature zone, three per Planktotron) consists of a pump (Lowara Ecocirc basic, Xylem Water Solutions, Fellbach, Germany), a membrane expansion vessel (MAG; HYDRO PLUS INOX, ZILMET, Wenden-Gerlingen, Germany), a boiler safety group (KSG; KSG 30 N, Watts Industries, Landau, Germany), a micro-bubble deaerator (Zeparo ZUV 22, TA

Heimeier, Erwitte, Germany), a fill and flush valve (FSA; Regusol, Oventrop, Olsberg, Germany), a fill and drain valve (KFE; KFE Ball Valve, Simplex, Argenbühl - Eisenharz, Germany), and a plate heat exchanger (WP2-20-DUO, ITB, Radolfzell, Germany). The plate heat exchanger of each pump circuit (temperature zone) consists of two primary compartments (for general heating or cooling) and one secondary compartment connected to the pillow plates of each temperature zone. The primary compartments are connected to the primary heating and the primary cooling circuit and regulated via a combination of a thermostatic valve and a thermally driven actuator. The primary heating circuit consists of a pump connected to a wall-hung gas boiler (turboTEC plus, Vaillant, Remscheid, Germany) for heating and a compact water chiller (weco 24 WBTX, gwk Gesellschaft Wärme Kältetechnik mbH, Meinerzhagen, Germany) for cooling. Using this system, the temperature zones can be adjusted between 5°C and 35°C.

To avoid wall growth during the experiments a rotating paddle with silicone lips was installed. It is driven by a brushless DC motor (BLF5120C-200FR and driver package, Oriental Motors, Tokyo, Japan) with a speed control range of 80–4000 r min<sup>-1</sup>. Due to transmission steps (gear box 200 : 1 and belt drive 3 : 1), the actual paddle speed can be regulated between 0.14 and 6.6 rotations min<sup>-1</sup>. The paddle is screwed (via a flange connection) on a slewing ring bearing (PRT-01-100-TO-AT10, igus GmbH, Köln, Germany), which is attached (with the motor) to a bridge and mounted on the top of the Planktotron. On top of the stub shaft (flange above the bearing), a slip ring (SC104-06-A01, LTN Servotechnik, Otterfing, Germany) transfers the data from the PT100 temperature sensors to the microcontroller board. The paddle itself can easily be disconnected and thus modified or shortened for other experimental setups (e.g., addition of sediment at the bottom of the Planktotrons).

The mesocosms can be continuously mixed by thermic convection through elevated temperature at the bottom, by speeding up the rotating paddle for a short time period, or by manual mixing with a disc (Striebel et al. 2013). Mixing the mesocosms by thermic convection or manually by disc disrupts the temperature stratification and prevents sedimentation, whereas speeding up the rotating paddle influences stratification only minimally. Sampling at different depths (e.g., in a stratified water column) is possible by connecting a sampling lance to one of the six sampling ports, which are installed at various depths (0.01 m, 0.33 m, 0.56 m, 0.79 m, 1.01 m, and 1.13 m below the top margin, Fig. 1). Sampling can also be conducted from the top (e.g., for zooplankton).

Each Planktotron is covered with two semi-circular, 10 mm thick low-iron float glass plates (Pilkington Opti-White). Two fully controllable LED aquarium lighting units (IT2040 Evergrow) are placed on top of the glass plates of each Planktotron. Each unit contains 55 LEDs of eight different colors to adjust the emitted light spectrum to a near-



**Fig. 2.** Light spectrum of used LEDs light panels at 20 cm water depth averaged for all 12 Planktotrons (mean  $\pm$  SE). The relative irradiance refers to the total irradiance at this depth.

natural light spectrum (Fig. 2). The maximum light intensity directly below the water surface is 660  $\mu\text{mol Photons m}^{-2} \text{s}^{-1}$  and  $k = 0.92$ . Different (dimnable) programs can be set (dusk, full sun, and dawn) with customizable duration and intensity; one lighting unit consumes 120 W.

Additional ports (three each) to the left and the right side (0.29 m, 0.56 m, and 0.82 m below the top margin) allow connecting the Planktotrons among each other, which enables meta-community approaches (Logue et al. 2011) or the usage of automated water-pumping on-line sensor instrumentation.

Temperature can be logged continuously at three depths (see above) using the temperature sensors connected to the microcontroller board, which is equipped with a RS-485 interface (for sending data via a two wire multipoint communications network), a SD card (recording the data), and a battery backed clock. For now, other parameters cannot be logged but must be monitored manually using external measurement equipment.

### Experimental setup

The first experiment conducted in the Planktotrons was a freshwater experiment with an artificially assembled diverse phytoplankton community (Table 1). We studied effects of grazing by zooplankton on this phytoplankton community and reactions of both phyto- and zooplankton to an external disturbance in the form of addition of dissolved organic matter. In aquatic systems, the input of allochthonous and colored dissolved organic matter has effects on light conditions and nutrient concentrations and thereby on the whole food web (Evans et al. 2005; Bartels et al. 2012a,b; Mormul et al. 2012; Hansson et al. 2013). While colored dissolved organic matter (cDOM) is expected to change the light availability and climate in the water column with negative impact on phytoplankton photosynthesis (Kirk 2010), the additional organic energy supplied by the dissolved organic carbon can

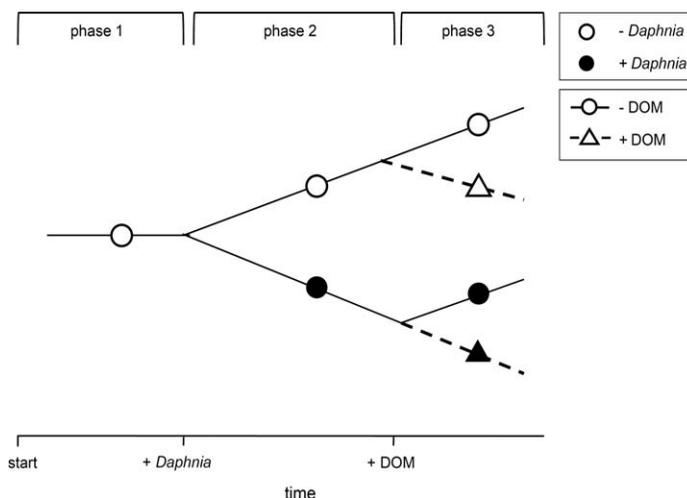
**Table 1.** Summary of phytoplankton species used in the experiment. After ciliate and *Daphnia* addition a contamination with two *Scenedesmus sp.* was reported in all Planktotrons (*Scenedesmus sp.* was used as food during pre-culturing). Biomass of *Scenedesmus sp.* was added to the group of (initially inoculated) Chlorophyceae for analyses.

Species	Group	Origin/ Strain number
<i>Chroococcus minutus</i>	Cyanophyceae	SAG 41.79
<i>Gymnodinium impatiens</i>	Dinophyceae	CCAC 0025
<i>Peridinium sp.</i>	Dinophyceae	SAG 2017
<i>Monoraphidium contortum</i>	Chlorophyceae	SAG 47.80
<i>Pediastrum duplex</i>	Chlorophyceae	SAG 84.80
<i>Carteria sp.</i>	Chlorophyceae	SAG 8-4
<i>Phacotus lenticularis</i>	Chlorophyceae	SAG 61-1
<i>Pinnularia neomajor</i>	Bacillariophyceae	SAG 2386
<i>Skeletonema subsalsum</i>	Bacillariophyceae	SAG 8.94
<i>Cryptomonas sp.</i>	Cryptophyceae	SAG 979-8
<i>Chroomonas sp.</i>	Cryptophyceae	SAG 980-1

lead to an increase in bacterial abundance (Hessen 1985; Tranvik 1988).

The experiment crossed the presence of a dominant zooplankton group (cladocerans) and the addition of dissolved organic material (DOM) in a  $2 \times 2$  factorial design (Fig. 3). The design was principally triplicated, yet the two treatments were applied at different times: zooplankton was added on day 22 while DOM addition started on day 72. Repeated measurements over time allowed to observe (1) effects of zooplankton at higher replication of  $n = 6$ , and (2) effects of DOM addition at presence/absence of zooplankton with  $n = 3$ , each in a before-after control-impact design.

In order to test the feasibility of using artificially assembled communities with specified composition of algae for such large volumes, we mixed 11 pre-cultured phytoplankton species from five taxonomic groups: Chlorophyceae, Dinophyceae, Cyanophyceae, Cryptophyceae, and Bacillariophyceae (see Table 1 for species composition). Cultures were obtained from various algal culture collections and pre-grown in WC growth medium (Guillard and Lorenzen 1972) under constant light ( $50 \mu\text{mol Photons m}^{-2} \text{s}^{-1}$ ) and temperature ( $18^\circ\text{C}$ ). We started the experiment by adding an identical inoculum with equal biovolume of each species to all Planktotrons. The composition of the medium was based on the WC growth medium, but nutrient concentrations were reduced by a 1 : 50 dilution (i.e.,  $33.6 \mu\text{g P L}^{-1}$ ,  $280.2 \mu\text{g N L}^{-1}$ ,  $41.9 \mu\text{g Si L}^{-1}$ ). During this first experiment, temperature was regulated by controlling room temperature, resulting in a mean temperature of  $18.7^\circ\text{C}$  and a temperature range between  $16^\circ\text{C}$  and  $21^\circ\text{C}$  during the entire experiment (with little variation among the Planktotrons,  $\text{SD} \pm 1.25^\circ\text{C}$ ). Light was supplied with a 16 : 8 h light : dark cycle using

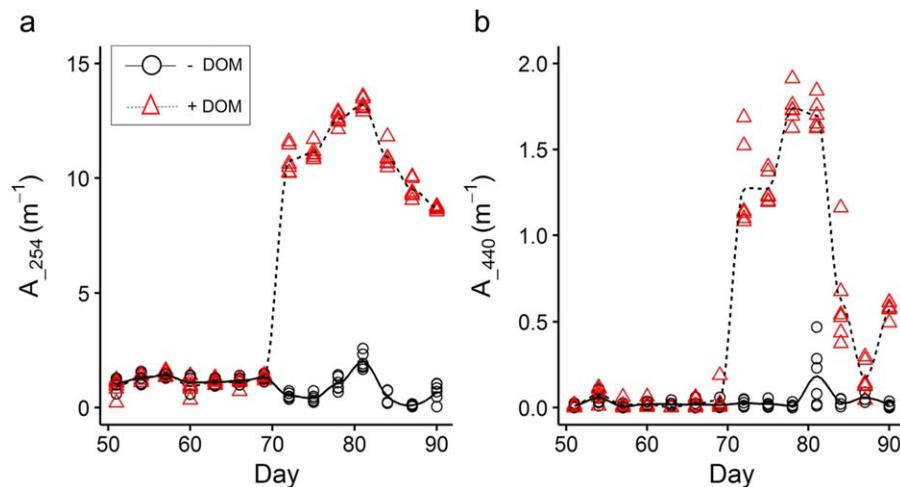


**Fig. 3.** Scheme of the experimental setup. In phase 1, the phytoplankton community was allowed to grow without any treatment. In phase 2, zooplankton was added to six of the Planktotrons. Dissolved organic matter (DOM) was added for phase 3 to six of the Planktotrons to obtain a  $2 \times 2$  factorial design. Empty symbols are used for treatments without *Daphnia* and filled symbols for treatments with *Daphnia* addition. Circles are used for treatments without DOM addition and triangles for treatments with dissolved organic matter addition. Solid lines are used for treatments without DOM addition and dashed lines are used for treatments with DOM addition.

LED lights as described above with full intensity. The paddle was set to 0.14 rotations per minute with the aim to not disturb zooplankton while being fast enough to prevent wall growth.

The experiment started in November 2014 and ran for 90 d in total (Fig. 3). Every third day 10% of the water was removed and replaced with fresh medium. The first 6 d were used as a pre-growth phase without exchange. After 22 d of phytoplankton growth (phase 1, Fig. 3), an additional trophic level was introduced for phase 2 of the experiment (Fig. 3): two ciliate species (*Stylonychia sp.* and *Euplotes daidaleos*), both effective feeders on microalgae (Finlay and Esteban 1998) were added to all Planktotrons. Simultaneously, three different *Daphnia* species were added to each of six Planktotrons (# 7–12): *Daphnia magna* (5 adults and 10 juveniles), *Daphnia pulex* (10 adults and 20 juveniles), and *Daphnia pulex* (10 adults and 20 juveniles). On day 72 phase 3 of the experiment started (Fig. 3) with the addition of 1000 mL solution of dissolved organic matter to six of the Planktotrons (#1, 3, 5, 7, 9, 11) to simulate a high discharge event with DOM input from terrestrial sources.

Terrigenous dissolved organic matter was produced by alkaline extraction (NaOH in deionized water at pH 12) of commercial peat for 48 h (Riedel et al. 2012). The peat slurry was filtered through a coarse sieve before passing through a series of  $3 \mu\text{m}$ ,  $1 \mu\text{m}$ , and finally  $0.2 \mu\text{m}$  large-volume filter cartridges (Causa-filter system, Infiltec GmbH, Germany) to remove bacteria and other particles. Lowest phosphate



**Fig. 4.** Absorption of DOM (**a**) at 254 nm (shown as decadal ( $\log_{10}$ ) absorption coefficient at 254 nm) and (**b**) at 440 nm (shown as decadal ( $\log_{10}$ ) absorption coefficient at 440 nm). Treatments without dissolved organic matter (DOM) are shown as black circles, those with DOM addition as red triangles. Solid and dashed lines represent the mean of the treatments after smoothing by local polynomial regression fitting without and with DOM addition, respectively.

content guided our choice of peat (Torfhumus Floragard<sup>®</sup>). The thereby produced deeply brown DOM-solution had total C content of  $901 \text{ mg L}^{-1}$ , and (decadal) absorption coefficients of  $10.2 \text{ mm}^{-1}$  and  $1.18 \text{ mm}^{-1}$  at wavelengths of 254 nm and 440 nm, respectively. We aimed at equal nutrient concentrations across treatments by adjusting the medium used alongside DOM. Still, this resulted in a higher phosphate concentration in treatments with than without DOM ( $19.05 \pm 3.32 \text{ } \mu\text{g P L}^{-1}$ , and  $13.94 \pm 1.97 \text{ } \mu\text{g P L}^{-1}$ , mean  $\pm$  SD, respectively). We observed no formation of precipitates upon adding the DOM solution to the Planktotrons. According to half-daily measurements of absorbance at 440 nm, colored DOM was lost at an exponential rate of approx.  $0.05 \text{ d}^{-1}$ . To prevent a decrease in DOM concentrations and to keep the light absorption at the level initially achieved by DOM addition, we therefore added further DOM (143 mL) on the four following sampling occasions (days 75, 78, 81, 84) after medium was exchanged. This counteracted dilution by sampling and degradation (Fig. 4). No DOM was added anymore over the last 6 d of the experiment.

### Sampling and analysis

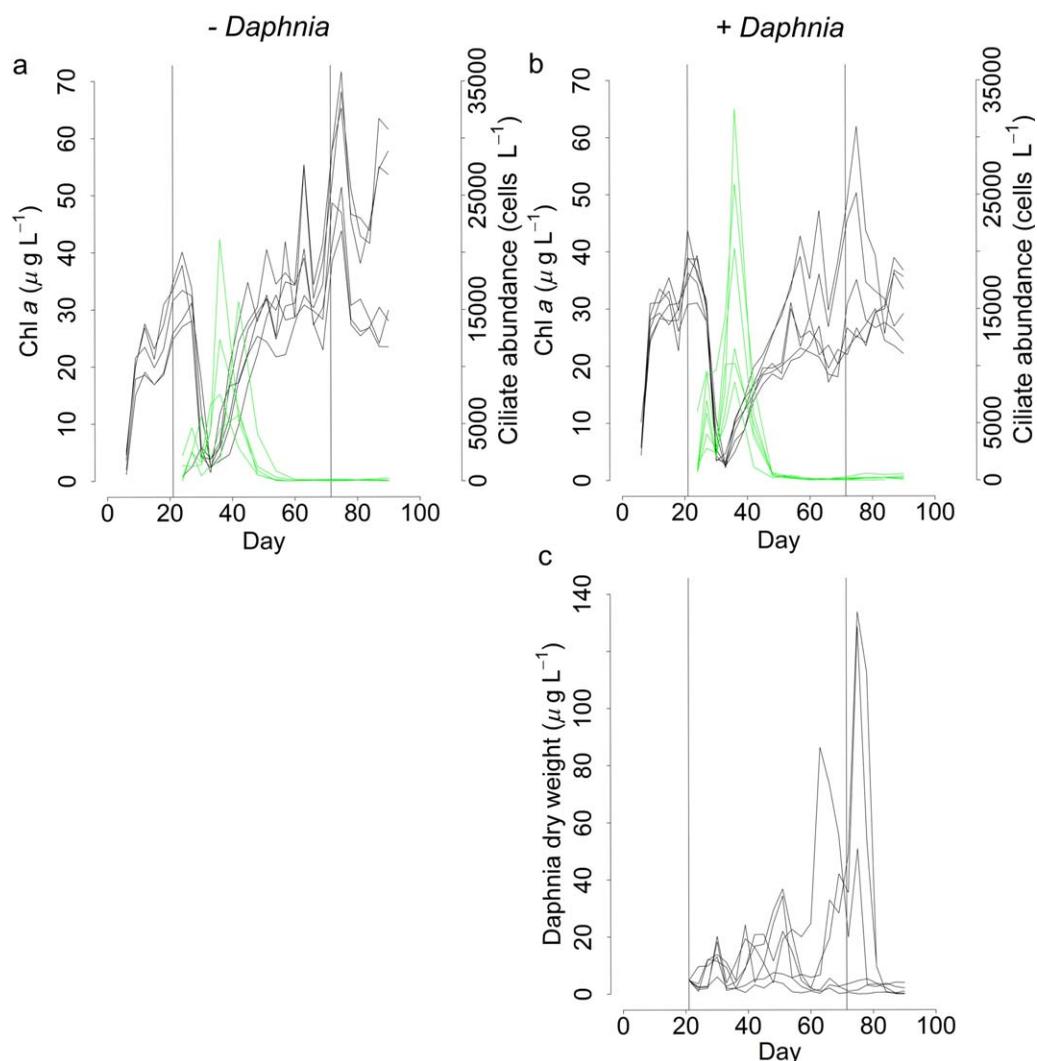
For sampling, an integrated water sample of 60 L (10% of the total volume) was used and subsequently replaced by new medium every third day. The removed water was filtered through a  $125 \text{ } \mu\text{m}$  mesh to remove zooplankton and then used for chemical and biological analyses (see below). In vivo chlorophyll *a* (Chl *a*) concentrations were measured using a hand-held fluorometer (TURNER DESIGNS, AquaFluor<sup>TM</sup>), which was calibrated using in vitro extracted Chl *a* samples and a Chl *a* standard (C5753 Sigma-Aldrich). Temperature and pH were measured with a pH-meter (SenTix<sup>®</sup>)

and  $\text{O}_2$  was measured with a single channel oxygen-meter (PreSens<sup>®</sup>).

Total phosphorus (TP) was quantified by persulfate digestion followed by molybdate reaction (Wetzel and Likens 2010). Soluble reactive phosphorus (SRP) and silicate (Si) were also quantified by molybdate reaction (Wetzel and Likens 2010). Samples for dissolved organic carbon (DOC) and for colored dissolved organic matter (cDOM) measurements were filtered with a double layer of pre-combusted and acid-washed glass-fiber filters (Whatman GF/F). The DOC samples were acidified to pH 2 with HCl (32%) and analyzed by high-temperature catalytic combustion (Shimadzu TOC-VCPH/CPN equipped with a TNM-1 module). CDOM samples were stored at  $4^\circ\text{C}$  in darkness pending analysis within 6 d on a Horiba Aqualog<sup>®</sup> spectrofluorometer to record absorption spectra (240–600 nm in steps of 3 nm).

Samples for determination of bacterial abundance were fixed with formaldehyde (final concentration 2.5%) and frozen at  $-80^\circ\text{C}$  until staining with SYBR gold, filtering on black polycarbonate filters ( $0.2 \text{ } \mu\text{m}$  GTBP), and direct counting using epifluorescence microscopy at 1000X magnification (Zeiss, Axio Scope A1).

Phytoplankton and protozoan samples for microscopic counts were fixed with Lugol's iodine (1% final concentration) and counted using an inverted microscope (Zeiss, Axiovert 10) at 400X magnification (Utermöhl 1958). For phytoplankton at least 400 cells per species were counted, cell volumes were calculated by geometrical approximation (Hillebrand et al. 1999). For ciliate counts, the samples were transferred to 100 mL sedimentation chambers, and the whole sample was counted. Zooplankton samples were counted immediately after sampling and afterwards dried in pre-weighed tin cups to determine dry weight.



**Fig. 5.** Phytoplankton biomass (black lines, Chl *a* in  $\mu\text{g L}^{-1}$ ) and ciliate abundance (green lines, individuals  $\text{L}^{-1}$ ) in (a) Planktotrons #1–6 without *Daphnia* addition and (b) Planktotrons #7–12 with *Daphnia* addition. Lines indicate the different Planktotrons. The first vertical line represents the ciliate/zooplankton addition and the second vertical line represents the dissolved organic matter (DOM) addition. Differentiation by DOM treatments during the third phase is not included in this figure. Data for one Planktotron (Chl *a* concentrations until day 21 and ciliate abundances until day 66) are missing.

### Statistics

All statistical procedures and graphs were performed using R version 3.3.1 (R Core Team 2016) using the packages plyr, Hmisc, scales, lsr, vegan, ez, ggplot2, ggthemes, compact, and grid. Analyses were done separately for phase 2, when protozoa were added to all systems as well as *Daphnia* were added to six Planktotrons, and for phase 3, when also DOM was added. Treatment effects on phytoplankton biomass, phytoplankton richness, phytoplankton evenness, ciliate abundance, and bacterial abundance were analyzed by repeated-measures ANOVA (rmANOVA) using the factorial design of treatments as contrasts between subjects (i.e., Planktotrons) and time as well as all treatment and time interactions within subjects. Homogeneity of variances

between factors was tested using the Bartlett test and eventually achieved after log-transformation. To test sphericity within factors, we used Mauchly's test and if significant applied Greenhouse-Geisser correction (Winer et al. 1991).

In order to compare the level of control in the Planktotrons, we compared the between-replicate variation in our experiment to the between-replicate variation in other experimental approaches. From our experiment, we calculated the coefficient of variation (CV) of Chl *a* concentration for each sampling day for each unique treatment combination. We aggregated these to mean CV ( $\pm$  standard deviation) for each of the three experimental phases. As a comparison, we used between-replicate CVs for > 756 experiments of phytoplankton responses to fertilization and grazing (using the

**Table 2.** Results from rmANOVA for day 24 to day 69 (phase 2, after *Daphnia* addition and before dissolved organic matter (DOM) addition). Ciliate abundances were log-transformed; no data was available for one Planktotron. Degrees of freedom numerator (dfN), degrees of freedom denominator (dfD), and *F*-values for each test are depicted, as well as *p*-values in brackets. Effects significant at  $p < 0.05$  are highlighted in bold.

Factor	Chl <i>a</i>				Log(ciliates)				Phytoplankton richness				Phytoplankton evenness			
	dfN	dfD	<i>F</i>	<i>p</i>	dfN	dfD	<i>F</i>	<i>p</i>	dfN	dfD	<i>F</i>	<i>p</i>	dfN	dfD	<i>F</i>	<i>p</i>
<i>Between subjects</i>																
<i>Daphnia</i>	1	10	1.7	0.22	1	9	<b>6.06</b>	<b>&lt; 0.05</b>	1	10	1.23	0.29	1	10	<b>7.02</b>	<b>&lt; 0.05</b>
<i>Within subjects</i>																
Time	14	140	<b>50.22</b>	<b>&lt; 0.001</b>	9	81	<b>18.76</b>	<b>&lt; 0.001</b>	8	80	<b>6.4</b>	<b>&lt; 0.001</b>	8	80	<b>25.32</b>	<b>&lt; 0.001</b>
Time × <i>Daphnia</i>	14	140	<b>3.35</b>	<b>&lt; 0.001</b>	9	81	2.67	0.10	8	80	0.69	0.7	8	80	<b>2.69</b>	<b>&lt; 0.05</b>

ELSIE database underlying (Elser et al. 2007; Gruner et al. 2008). We did so separately for field experiments (similar size as Planktotrons,  $N = 570$ ) and lab experiments (similar level of control,  $N = 186$ ).

## Assessment

### Population dynamics and interactions between different trophic levels (phase 1 and 2)

Phytoplankton biomass (measured as Chl *a* concentration) increased in all Planktotrons (Fig. 5a,b) until the addition of ciliates (at day 21 to all Planktotrons) and *Daphnia* (to half of the Planktotrons, Fig. 5b). Subsequently, ciliate abundance strongly increased until days 35–40 (Fig. 5a,b), when it formed a single peak that was higher with than without *Daphnia* (Table 2). The grazing by ciliates decreased Chl *a* concentrations and ciliate abundance then dropped in all Planktotrons (Fig. 5a,b). Afterwards phytoplankton biomass increased again, more pronounced in the treatments without *Daphnia*. *Daphnia* biomass (Fig. 5c) increased slowly and—in contrast to ciliates—could only weakly curb phytoplankton development compared to the treatments without *Daphnia* (Table 2). On day 75 *Daphnia* biomass reached its maximum and thereafter declined below initial values (Fig. 5c).

Phytoplankton richness changed over time and phytoplankton evenness was affected by the presence of *Daphnia* (Table 2). The composition of phytoplankton communities changed strongly over time in all Planktotrons (Fig. 6). Cryptophytes increased at the beginning of the experiment and dominated the phytoplankton communities in all Planktotrons likewise. After ciliates and daphnids were added, the relative amount of cryptophytes decreased while chlorophytes increased and eventually dominated all phytoplankton communities. In two of the Planktotrons cyanobacteria increased shortly after zooplankton addition and replaced the chlorophytes in these Planktotrons. All other functional groups (Dinophyceae, Bacillariophyceae) had a share of less than 6% during the experiment.

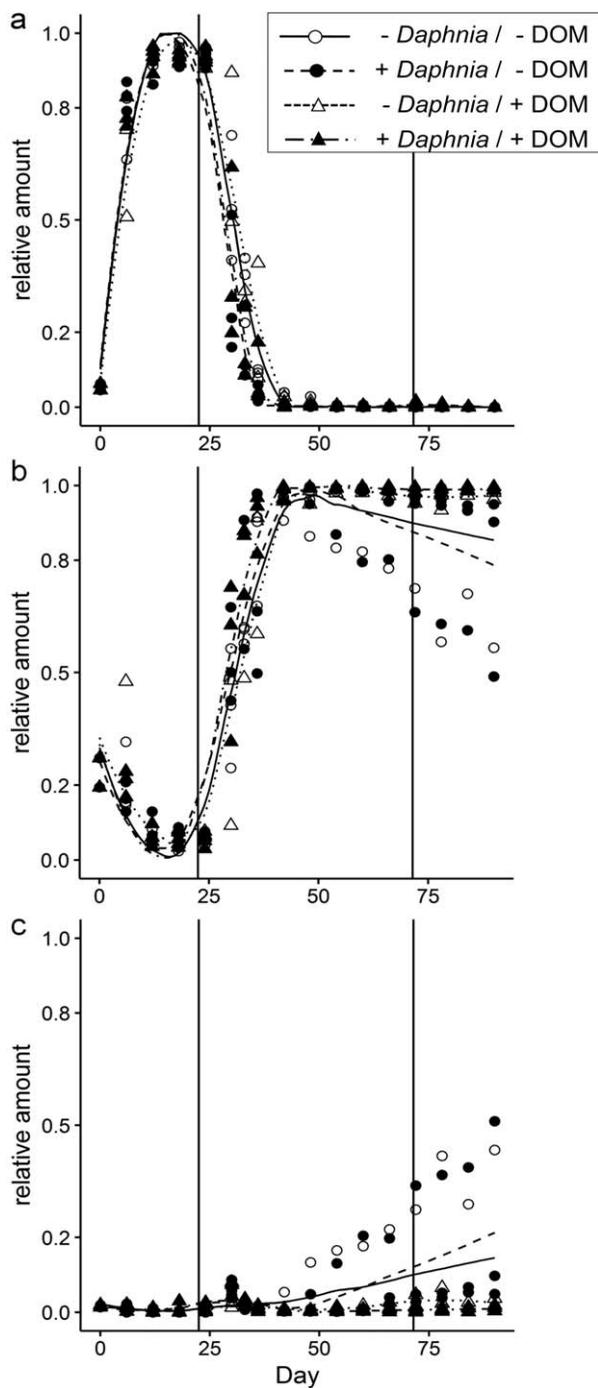
### Effects of adding dissolved organic matter in phase 3

To investigate the effect of DOM on abundances of bacteria and ciliates, as well as biomass of phytoplankton and *Daphnia*, we analyzed the data of phase 3 following DOM addition (Fig. 7). The addition of DOM at day 72 caused a strong change in light climate in the treated Planktotrons (Fig. 4) and supplied an additional amount of DOC of  $1.5 \text{ mg L}^{-1}$  principally available to microbial consumers. The addition of DOM into half of the Planktotrons prompted a strong and fast increase of bacterial abundance (Table 3; Fig. 7a,b) followed by a slow gradual decrease until the end of the experiment. The used DOM was highly bioavailable as only moderate increases of DOC could be measured in the Planktotrons; comparing data from pre- to post-DOM addition sampling occasions revealed an increase of  $0.82$  vs.  $0.48 \text{ mg L}^{-1}$  in treated vs. non-treated Planktotrons, respectively.

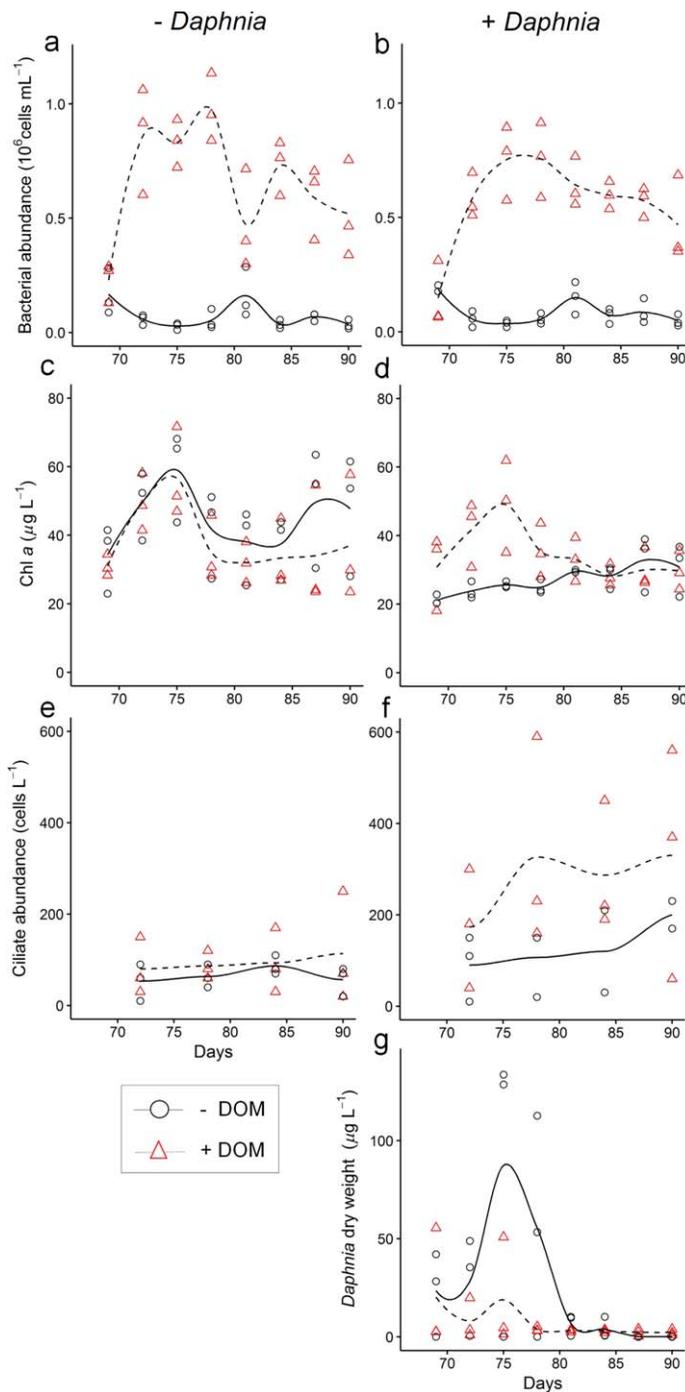
Total phytoplankton biomass was not significantly affected by *Daphnia* nor DOM addition in phase 3, however both led to significantly different developments over time (Table 3). Phytoplankton species richness and evenness were not significantly affected by *Daphnia* nor DOM addition during the third phase of the experiment (Table 3). DOM addition did not significantly affect ciliate abundance in the Planktotrons (Table 3; Fig. 7e,f). However, ciliate abundance was marginally higher in all treatments with *Daphnia* addition during the third phase. Finally, the addition of DOM affected *Daphnia*, and reduced its dry weight (Fig. 7g).

### Comparability of the indoor mesocosms with laboratory and field experiments

The between-replicate variation in phytoplankton biomass in the Planktotrons was well within the range of published laboratory and field experiments (Fig. 8). During phase 1, when no treatment had been applied yet, the mean variation among replicate Planktotrons was lower than the average from other plankton experiments. In phase 2 and 3, treatments with *Daphnia* addition remained at very low between replicate variation, indicating parallel dynamics in the replicates. Without *Daphnia* addition, the CV increased,



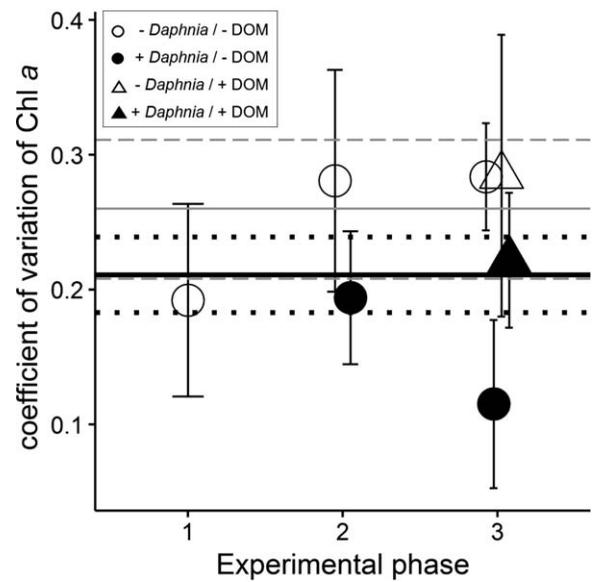
**Fig. 6.** Phytoplankton species composition, calculated as relative amounts of functional groups for each Planktoton: (a) Cryptophytes, (b) Chlorophytes, (c) Cyanobacteria. Circles/triangles are used for treatments without/with dissolved organic matter (DOM), respectively. Filled/open symbols display treatments with/without *Daphnia* addition, respectively. Lines are daily mean values smoothed by local polynomial regression fitting. Data for one Planktoton are missing. The two vertical lines represent the moments of ciliate/zooplankton addition (1<sup>st</sup> line) and of DOM addition (2<sup>nd</sup> line).



**Fig. 7.** Phase 3 of the experiment. Bacterial abundances (cells mL<sup>-1</sup>) over time (a) Planktoton #1–6, without *Daphnia*, (b) Planktoton #7–12, with *Daphnia*; Phytoplankton biomass (Chl *a* in µg L<sup>-1</sup>) over time for (c) Planktoton #1–6, without *Daphnia* (d) Planktoton #7–12, with *Daphnia*; Ciliate abundance (cells per L<sup>-1</sup>) over time for (e) Planktoton #1–6, without *Daphnia*; (f) Planktoton #7–12, with *Daphnia*; (g) *Daphnia* abundance for Planktoton #7–12 (dry weight µg L<sup>-1</sup>). Black circles/red triangles are used for treatments without/with dissolved organic matter (DOM). The black solid/dashed line represents the mean of the treatments without/with DOM addition smoothed by local polynomial regression fitting.

**Table 3.** Results from rmANOVA for day 72 to day 90 (phase 3, with *Daphnia* and dissolved organic matter (DOM) addition). Bacteria abundance, phytoplankton Chl *a* concentration and ciliate abundance were log-transformed. Degrees of freedom numerator (dfN), degrees of freedom denominator (dfD), and *F*-values for each test are shown, as well as *p*-values in brackets. Effects significant at  $p < 0.05$  are highlighted in bold.

Factor	Log(bacteria)			Log(Chl <i>a</i> )			Log(ciliates)			Phytoplankton richness			Phytoplankton evenness								
	dfN	dfD	<i>F</i>	<i>p</i>	dfN	dfD	<i>F</i>	<i>p</i>	dfN	dfD	<i>F</i>	<i>p</i>	dfN	dfD	<i>F</i>	<i>p</i>					
<i>Between subjects</i>																					
<i>Daphnia</i>	1	8	0.73	0.42	1	8	4.30	0.072	1	8	4.06	0.08	1	8	1.19	0.31	1	8	1.98	0.2	
DOM	1	8	<b>141.3</b>	<b>&lt;0.001</b>	1	8	0.02	0.89	1	8	2.42	0.16	1	8	0.01	0.92	1	8	0.24	0.63	
<i>Daphnia</i> × DOM	1	8	1.17	0.31	1	8	1.78	0.22	1	8	1.18	0.31	1	8	0.48	0.51	1	8	0.004	0.95	
<i>Within subjects</i>																					
Time	7	56	<b>11.75</b>	<b>&lt;0.001</b>	7	56	<b>17.41</b>	<b>&lt;0.001</b>	3	24	2.68	0.07	3	24	0.74	0.54	3	24	1.25	0.31	
Time × <i>Daphnia</i>	7	56	1.73	0.2	7	56	<b>4.07</b>	<b>&lt;0.05</b>	3	24	1.32	0.29	3	24	1.29	0.3	3	24	1.19	0.33	
Time × DOM	7	56	<b>29.94</b>	<b>&lt;0.001</b>	7	56	<b>6.03</b>	<b>&lt;0.01</b>	3	24	1	0.41	3	24	1.16	0.35	3	24	2.61	0.07	
Time × <i>Daphnia</i> × DOM	7	56	1.89	0.17	7	56	1.42	0.27	3	24	0.72	0.55	3	24	0.6	0.62	3	24	0.95	0.43	



**Fig. 8.** Comparison of between replicate variation in the Planktotrons to other experimental studies. Based on literature data, the between replicate variance in plankton experiments was expressed as coefficient of variation (CV) for each study. The solid lines represent the average CV for field experiments (black, 570 studies) and lab experiments (gray, 186 studies), the dashed lines the corresponding 95% confidence intervals of the CV. Compared to this, we plotted the mean CV and its SD of Chl *a* concentrations within the different treatments in our experiment. Open symbols are used for treatments without *Daphnia* addition and filled symbols for treatments with *Daphnia* addition. Circles are used for treatments without dissolved organic matter (DOM) and triangles for treatments with dissolved organic matter addition.

but remained within the range of CV derived from other laboratory and field experiments.

### Discussion

#### Technical feasibility

The Planktotrons allow performing long-term and large-scale mesocosm experiments with natural and artificial communities under highly controlled conditions. One of the most relevant disadvantages often coming along with mesocosms experiments is the biomass growth on walls after a few weeks of experimental duration (Chen et al. 1997; Sommer et al. 2006). The rotating paddles with silicon lips installed in the Planktotrons successfully removed particles from the walls despite relatively slow rotation speed (8.23 rotations per hour) to prevent harming plankton. We did not observe any wall growth over an extended experimental time of 90 d. McCauley et al. (1999) set up a long-term experiment using 20-L vessels, where they daily scraped the walls manually to prevent periphyton growth. This effort can be considered as effective as our continuously rotating paddle, yet it is not appropriate for larger and deeper mesocosms.

An additional advantage of the Planktotron facility is the extensive control of temperature and light conditions. In the

here described pilot experiment temperature was regulated by controlling room temperature, resulting in a temperature range between 16°C and 21°C during the entire experiment. Other experiments meanwhile made full use of the temperature regulation system and achieved very small temperature deviation (L. Verbeek et al., unpubl.; Elías 2016). The light intensity, the duration of the light period as well as the light spectrum can be manipulated, extending the range of possible experiments in the Planktotrons. In this experiment, we used the entire available light spectrum and maximal light intensity, which is higher than commonly used in other indoor-facilities (Verschoor et al. 2003; Peperzak et al. 2011).

The Planktotrons offer the possibility to manipulate existence and community composition of entire trophic levels, allowing new approaches to investigate them separately as well as their interaction. In this experiment, species from different trophic levels were used, namely 11 different phytoplankton species, two different ciliate species, and three different *Daphnia* species. Additionally, bacteria, which were not separately inoculated, also developed and readily reacted to DOM addition. Thus, our experiment included four established trophic levels, whose individual responses to abiotic as well as biotic treatments could be investigated at the level of individual species and communities.

One aim of the first experiment conducted in the Planktotrons was to establish realistic population sizes and to obtain population dynamics in the Planktotrons. The phytoplankton biomass (measured as Chl *a* concentration) in this experiment was in a range reported for other systems under oligotrophic to mesotrophic conditions (Beaver and Crisman 1989). The ciliate abundances measured in our experiment were typical for oligotrophic systems (Beaver and Crisman 1989). As we did not specifically inoculate the Planktotrons with bacteria, these could only originate from the (non-axenic) phytoplankton cultures. This might have been the reason why we found very low (without DOM addition) and only slightly higher bacterial abundances (with DOM addition) in the Planktotrons, while natural abundances in lakes are often two orders of magnitude higher (Pace and Cole 1996; Pernthaler et al. 1998). The population sizes of *Daphnia* in this experiment were also lower compared to field observations (DeMott and Gulati 1999). We attribute this to the low initial abundances of only 75 individuals per Planktotron, which probably suffered from the inoculation process. Obviously, the population could not reach realistic, naturally occurring abundances. Nevertheless, we observed population dynamics comparable to lakes (Scheffer et al. 1997) and laboratory experiments (Becks et al. 2012).

The design of the Planktotrons offers the possibility to set up a semi-continuous system, which is routinely used in laboratory experiments, but not feasible in outdoor mesocosms. The Planktotrons can be filled easily with freshwater and/or marine water by a pipe. Thus, any amount of water can be exchanged daily if necessary, although the frequency and

the amount should be adapted to the inoculated organisms. Growth rates of marine phytoplankton, for example, are reported to be typically in the range of either  $< 0.5$  doublings per day ( $\mu = 0.35 \text{ d}^{-1}$ ) or  $> 1.0$  doubling per day ( $\mu = 0.69 \text{ d}^{-1}$ ) (Goldman et al. 1979). Contrary to chemostat experiments, where the dilution rates could be more than  $1.0 \text{ d}^{-1}$  (Sommer 1983; Fussmann et al. 2000), we suggest a dilution rate not higher than  $0.3 \text{ d}^{-1}$  as also used in other semi-continuous laboratory approaches (Flöder et al. 2002). In this experiment, 10% of the medium was replaced every third day, which corresponds to a dilution rate of approximately  $0.03 \text{ d}^{-1}$ . This dilution rate exactly replaced our sampled water volume. It was lower than in laboratory experiments, yet sufficiently replenishing removed nutrients for phytoplankton growth and beneficially little interfering with the zooplankton.

The large size of the Planktotrons (600 L) allows large sample volumes for a simultaneous determination of several volume-intense measures such as pigments and fatty acids. Every third day, 60 L were removed and replaced with fresh medium. From these 60 L not even 10 L were used for analyses such as counting (bacteria, ciliates, and phytoplankton), C : N : P analyses, dissolved nutrients and dissolved organic carbon analyses, and pigment and fatty acid determination. Consequently, such analyses could be done every day without harmful system interruption.

The level of control of the Planktotrons proved satisfactory, as the among-replicate coefficient of variation in biomass did not markedly differ from other field or laboratory experiments, although these were mostly conducted with much smaller volumes. Especially with zooplankton, the between-replicate variation in the Planktotrons was at the lower range reported from other studies—or even below that (Conquest 1983). More comparisons of results from future experiments conducted in the Planktotrons with field data and whole ecosystem setups may allow derivation of scaling strategies to accurately extrapolate the results to whole ecosystems (Schindler 1998). This seems especially relevant as the Planktotrons are designed for multi-trophic experiments, yet likely will not be suitable to host higher predators like fish as achieved in a mesocosm study by Harrass and Taub (1985), for instance. Full consideration of top-down ecological control, i.e., predator effects, in mesocosm environments will remain to be a challenge, especially for marine environments, yet mechanisms of planktonic interactions identified in systems like the Planktotrons are key to ecosystem functioning of pelagic environments from ponds to the oceans and may be fruitfully considered in a larger and (more) natural context.

## Experiment

The sequence of ciliate, *Daphnia* and DOM addition resulted in strong consumer-prey dynamics. First, phytoplankton increased until ciliates were added. Afterwards phytoplankton decreased, but could recover again after ciliates

decreased. The ciliates were added to all Planktotrons at the same time as *Daphnia* were added to half of them. The ciliates, however, reached their peak before *Daphnia* did, most probably due to the differing growth rates: While the maximum growth rate of ciliates is close to  $3 \text{ d}^{-1}$ , equivalent to approximate 4 cell divisions  $\text{d}^{-1}$  (Weisse 2006), *Daphnia* has a maximum population growth rate of about  $0.7 \text{ d}^{-1}$  under optimal conditions (Mitchell and Lampert 2000). Thus, the ciliates efficiently grazed down the phytoplankton to drastically reduced abundances with negative feedback on their own abundance. Phytoplankton communities were dominated by cryptomonads before ciliate addition (cryptomonad biovolume on day 18 before ciliate addition:  $2.1 \times 10^7 \mu\text{m}^3 \text{ mL}^{-1} \pm 0.68 \times 10^7 \mu\text{m}^3 \text{ mL}^{-1}$ , mean  $\pm$  SD), which could be ingested by algivorous ciliates such as *Euplotes* (Fenchel 1980). This ciliate can achieve clearing rates between  $2 \times 10^{-4}$  and  $1 \times 10^{-3} \text{ mL h}^{-1}$  depending on cell volume (Fenchel 1980). Thus, at the beginning of the experiment, the ciliate populations were the more efficient grazers compared to the small *Daphnia* populations. Interestingly, *Daphnia* was not capable of reducing ciliate abundance although they could feed on the ciliates we used (Burns 1968). Even after the ciliate abundances declined, *Daphnia* could not reach high abundances. In our experiment, *Daphnia* reached dry weights not higher than  $133.7 \mu\text{g L}^{-1}$ , which is distinctly less than the  $1500 \mu\text{g L}^{-1}$  reported for natural systems under optimal conditions (Evans et al. 1995). A possible explanation might be the relatively small amount of inoculated *Daphnia* at the beginning of the experiment. With small population size, stochastic variations and the probability that fluctuations will lead to extinction is high. *Daphnia* were added with abundances of 15 individuals per Planktotron of *D. magna* and 30 individuals per Planktotron for *D. pulex* and *D. pulicaria*. Additionally, water exchange and sampling were conducted every third day; therefore, the population was kept low and the population development was impaired. Furthermore, a few days before the *Daphnia* abundances decreased suddenly in this first experiment, a fire in a nearby steel-enterprise occurred and wads of smoke passed the experimental facility. This might also have negatively influenced the *Daphnia* population in our experiment. Nevertheless, the abundances of *Daphnia* were appropriate to decrease the phytoplankton biomass and affected the lower trophic level after ciliate abundances were reduced. A second experiment conducted in the Planktotrons (A. Gall, unpubl.) under comparable conditions (rotation speed, mixing device, nutrient addition, light conditions), but with higher initial abundances of *Daphnia* (about four times higher) showed that *Daphnia* were able to increase their biomass significantly over time (90 d) and reached abundances of up to 50 individuals per liter.

In addition to the zooplankton treatment, we applied a second factor, the addition of terrigenous colored dissolved organic matter (DOM) as a naturally occurring (chemical)

disturbance in aquatic systems. The input of allochthonous DOM can affect the whole food web because it is supposed to simultaneously change the availability of light and nutrients (Bartels et al. 2012a,b). While colored dissolved organic matter (cDOM) is expected to decrease the light intensity and to modify the light climate in the water column with all its negative impact on phytoplankton photosynthesis (Kirk 2010), the additionally available dissolved organic carbon can be used by bacteria and may thus lead to an increase in bacterial abundance (Hessen 1985; Tranvik 1988).

In our experiment, we found distinct effects of DOM on phytoplankton over time: Phytoplankton biomass was slightly (but not significantly) lower in the treatments with DOM addition compared to those without DOM addition, when no *Daphnia* were present. In contrast, phytoplankton biomass was higher with DOM addition compared to treatments without DOM addition, when *Daphnia* were present. DOM input can stimulate phytoplankton and bacterial production especially when nutrients are scarce (Guadayol et al. 2009; Pecqueur et al. 2011; Liess et al. 2015). As expected (Hessen 1985; Tranvik 1988) the effect of DOM addition on bacteria was positive, independently of the *Daphnia* treatment. As reasons for stimulating effects of DOM on phytoplankton past studies have speculated about nutrient mediated effects of DOM (Daggett et al. 2015; Liess et al. 2015). We are at present unable to explain why the DOM effect on phytoplankton may depend on presence of *Daphnia*, but are investigating grazing-induced changes of phytoplankton composition as potentially playing a role. In this experiment, DOM addition also increased the variance of most measured variables including phytoplankton community composition, which hampered the identification of simple explanatory mechanisms. The increased variance itself may simply be attributed to the longer experimental time at the moment of DOM addition and concomitant drift effects.

### Comments and recommendations

The Planktotrons were built with the purpose to conduct freshwater and marine experiments using natural or artificial communities and offer a great range of potential experimental manipulations. Consequently, further experiments could test various consequences of climate change such as temperature change, changes in nutrient conditions, changes in  $\text{CO}_2$  concentrations, and effects of DOM increase on natural phytoplankton-zooplankton communities as well as on benthic communities. Interactions between benthic and planktonic communities could also be investigated. Furthermore, this facility offers the possibility to conduct multispecies ecotoxicity tests of various chemicals; allowing the investigation of responses of whole communities or of various trophic levels, or the study of interactive effects of various organisms (Taub 1997; Beyers 2012). The Planktotrons can be connected to set up meta-community studies. Until now, meta-

community studies were limited to small-scale experiments or observations in the field without manipulation (Logue et al. 2011). The large size of the Planktotrons does not only offer the possibility for large-volume samplings as described in this manuscript, but also for high-frequency samplings. High-frequency samplings are advantageous for organisms with a fast growth rate, such as bacteria. Hereby, samples can be taken hourly for multiple days, which is hardly possible in small-scale experiments or in the field. The possibility for large-volume and high-frequency sampling is particularly convenient in the context of theoretical models, which can then be developed and validated based on a large number of variables.

Indoor mesocosms are costly and space-consuming, therefore not every research institute can afford such approaches. The Planktotrons are a very new and unique indoor mesocosm set up, which offers various research possibilities. The research team of the ICBM is open for collaborations and offers the Planktotrons also for use by external researchers.

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#### Conflict of Interest

None declared.

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